

21437
Ser. No.
09/529,043

SEQUENCE LISTING

<110> Forschungszentrum Juelich GmbH

<120> Method for microbial production of amino acids of the aspartate and/or glutamate family and agents which can be used in said method

<130> 1

<140> 09/529,043

<141> 2000-04-03

<150> PCT/EP98/06210

<151> 1998-09-30

<160> 4

<170> PatentIn Ver. 2.1

<210> 1

<211> 3728

<212> DNA

<213> Corynebacterium glutamicum

<220>

<221> CDS

<222> (165)..(3587)

<223> pyruvate carboxylase

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ctatcacct tggcgggtctc ttgttgaaag gaataattac tcta gtg tcg act cac 176
Val Ser Thr His
1

aca tct tca acg ctt cca gca ttc aaa aag atc ttg gta gca aac cgc 224
Thr Ser Ser Thr Leu Pro Ala Phe Lys Lys Ile Leu Val Ala Asn Arg
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ggc gaa atc gcg gtc cgt gct ttc cgt gca gca ctc gaa acc ggt gca 272
Gly Glu Ile Ala Val Arg Ala Phe Arg Ala Ala Leu Glu Thr Gly Ala
25 30 35

gcc acg gta gct att tac ccc cgt gaa gat cgg gga tca ttc cac cgc 320



Ala Thr Val Ala Ile Tyr Pro Arg Glu Asp Arg Gly Ser Phe His Arg
 40 45 50

tct ttt gct tct gaa gct gtc cgc att ggt acc gaa ggc tca cca gtc 368
 Ser Phe Ala Ser Glu Ala Val Arg Ile Gly Thr Glu Gly Ser Pro Val
 55 60 65

aag gcg tac ctg gac atc gat gaa att atc ggt gca gct aaa aaa gtt 416
 Lys Ala Tyr Leu Asp Ile Asp Glu Ile Ile Gly Ala Ala Lys Lys Val
 70 75 80

aaa gca gat gcc att tac ccg gga tac ggc ttc ctg tct gaa aat gcc 464
 Lys Ala Asp Ala Ile Tyr Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala
 85 90 95 100

cag ctt gcc cgc gag tgt gcg gaa aac ggc att act ttt att ggc cca 512
 Gln Leu Ala Arg Glu Cys Ala Glu Asn Gly Ile Thr Phe Ile Gly Pro
 105 110 115

acc cca gag gtt ctt gat ctc acc ggt gat aag tct cgc gcg gta acc 560
 Thr Pro Glu Val Leu Asp Leu Thr Gly Asp Lys Ser Arg Ala Val Thr
 120 125 130

gcc gcg aag aag gct ggt ctg cca gtt ttg gcg gaa tcc acc ccg agc 608
 Ala Ala Lys Lys Ala Gly Leu Pro Val Leu Ala Glu Ser Thr Pro Ser
 135 140 145

aaa aac atc gat gag atc gtt aaa agc gct gaa ggc cag act tac ccc 656
 Lys Asn Ile Asp Glu Ile Val Lys Ser Ala Glu Gly Gln Thr Tyr Pro
 150 155 160

atc ttt gtg aag gca gtt gcc ggt ggt ggc gga cgc ggt atg cgt ttt 704
 Ile Phe Val Lys Ala Val Ala Gly Gly Gly Gly Arg Gly Met Arg Phe
 165 170 175 180

gtt gct tca cct gat gag ctt cgc aaa tta gca aca gaa gca tct cgt 752
 Val Ala Ser Pro Asp Glu Leu Arg Lys Leu Ala Thr Glu Ala Ser Arg
 185 190 195

gaa gct gaa gcg gct ttc ggc gat ggc gcg gta tat gtc gaa cgt gct 800
 Glu Ala Glu Ala Ala Phe Gly Asp Gly Ala Val Tyr Val Glu Arg Ala
 200 205 210

gtg att aac cct cag cat att gaa gtg cag atc ctt ggc gat cac act 848
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 215 220 225

gga gaa gtt gta cac ctt tat gaa cgt gac tgc tca ctg cag cgt cgt 896

Gly Glu Val Val His Leu Tyr Glu Arg Asp Cys Ser Leu Gln Arg Arg
 230 235 240

cac caa aaa gtt gtc gaa att gcg cca gca cag cat ttg gat cca gaa 944
 His Gln Lys Val Val Glu Ile Ala Pro Ala Gln His Leu Asp Pro Glu
 245 250 255 260

ctg cgt gat cgc att tgt gcg gat gca gta aag ttc tgc cgc tcc att 992
 Leu Arg Asp Arg Ile Cys Ala Asp Ala Val Lys Phe Cys Arg Ser Ile
 265 270 275

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 Gly Tyr Gln Gly Ala Gly Thr Val Glu Phe Leu Val Asp Glu Lys Gly
 280 285 290

aac cac gtc ttc atc gaa atg aac cca cgt atc cag gtt gag cac acc 1088
 Asn His Val Phe Ile Glu Met Asn Pro Arg Ile Gln Val Glu His Thr
 295 300 305

gtg act gaa gaa gtc acc gag gtg gac ctg gtg aag gcg cag atg cgc 1136
 Val Thr Glu Glu Val Thr Glu Val Asp Leu Val Lys Ala Gln Met Arg
 310 315 320

ttg gct gct ggt gca acc ttg aag gaa ttg ggt ctg acc caa gat aag 1184
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 325 330 335 340

atc aag acc cac ggt gca gca ctg cag tgc cgc atc acc acg gaa gat 1232
 Ile Lys Thr His Gly Ala Ala Leu Gln Cys Arg Ile Thr Thr Glu Asp
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cca aac aac ggc ttc cgc cca gat acc gga act atc acc gcg tac cgc 1280
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 Ser Pro Gly Gly Ala Gly Val Arg Leu Asp Gly Ala Ala Gln Leu Gly
 375 380 385

ggc gaa atc acc gca cac ttt gac tcc atg ctg gtg aaa atg acc tgc 1376
 Gly Glu Ile Thr Ala His Phe Asp Ser Met Leu Val Lys Met Thr Cys
 390 395 400

cgt ggt tcc gac ttt gaa act gct gtt gct cgt gca cag cgc gcg ttg 1424
 Arg Gly Ser Asp Phe Glu Thr Ala Val Ala Arg Ala Gln Arg Ala Leu
 405 410 415 420

gct gag ttc acc gtg tct ggt gtt gca acc aac att ggt ttc ttg cgt 1472

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Ala Leu Leu Arg Glu Glu Asp Phe Thr Ser Lys Arg Ile Ala Thr Gly	
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ttc att gcc gat cac ccg cac ctc ctt cag gct cca cct gct gat gat	1568
Phe Ile Ala Asp His Pro His Leu Leu Gln Ala Pro Pro Ala Asp Asp	
455	460 465
gag cag gga cgc atc ctg gat tac ttg gca gat gtc acc gtg aac aag	1616
Glu Gln Gly Arg Ile Leu Asp Tyr Leu Ala Asp Val Thr Val Asn Lys	
470	475 480
cct cat ggt gtg cgt cca aag gat gtt gca gct cct atc gat aag ctg	1664
Pro His Gly Val Arg Pro Lys Asp Val Ala Ala Pro Ile Asp Lys Leu	
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cct aac atc aag gat ctg cca ctg cca cgc ggt tcc cgt gac cgc ctg	1712
Pro Asn Ile Lys Asp Leu Pro Leu Pro Arg Gly Ser Arg Asp Arg Leu	
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aag cag ctt ggc cca gcc gcg ttt gct cgt gat ctc cgt gag cag gac	1760
Lys Gln Leu Gly Pro Ala Ala Phe Ala Arg Asp Leu Arg Glu Gln Asp	
520	525 530
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Ala Leu Ala Val Thr Asp Thr Thr Phe Arg Asp Ala His Gln Ser Leu	
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Leu Ala Thr Arg Val Arg Ser Phe Ala Leu Lys Pro Ala Ala Glu Ala	
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Val Ala Lys Leu Thr Pro Glu Leu Leu Ser Val Glu Ala Trp Gly Gly	
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Ala Thr Tyr Asp Val Ala Met Arg Phe Leu Phe Glu Asp Pro Trp Asp	
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agg ctc gac gag ctg cgc gag gcg atg ccg aat gta aac att cag atg	2000
Arg Leu Asp Glu Leu Arg Glu Ala Met Pro Asn Val Asn Ile Gln Met	
600	605 610
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Val Cys Arg Ala Phe Val Lys Glu Ala Ala Ser Ser Gly Val Asp Ile	
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ttc cgc atc ttc gac gcg ctt aac gac gtc tcc cag atg cgt cca gca	2144
Phe Arg Ile Phe Asp Ala Leu Asn Asp Val Ser Gln Met Arg Pro Ala	
645	650 655 660
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Ile Asp Ala Val Leu Glu Thr Asn Thr Ala Val Ala Glu Val Ala Met	
665	670 675
gct tat tct ggt gat ctc tct gat cca aat gaa aag ctc tac acc ctg	2240
Ala Tyr Ser Gly Asp Leu Ser Asp Pro Asn Glu Lys Leu Tyr Thr Leu	
680	685 690
gat tac tac cta aag atg gca gag gag atc gtc aag tct ggc gct cac	2288
Asp Tyr Tyr Leu Lys Met Ala Glu Glu Ile Val Lys Ser Gly Ala His	
695	700 705
atc ttg gcc att aag gat atg gct ggt ctg ctt cgc cca gct gcg gta	2336
Ile Leu Ala Ile Lys Asp Met Ala Gly Leu Leu Arg Pro Ala Ala Val	
710	715 720
acc aag ctg gtc acc gca ctg cgc cgt gaa ttc gat ctg cca gtg cac	2384
Thr Lys Leu Val Thr Ala Leu Arg Arg Glu Phe Asp Leu Pro Val His	
725	730 735 740
gtg cac acc cac gac act gcg ggt ggc cag ctg gca acc tac ttt gct	2432
Val His Thr His Asp Thr Ala Gly Gly Gln Leu Ala Thr Tyr Phe Ala	
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Ala Ala Gln Ala Gly Ala Asp Ala Val Asp Gly Ala Ser Ala Pro Leu	
760	765 770
tct ggc acc acc tcc cag cca tcc ctg tct gcc att gtt gct gca ttc	2528
Ser Gly Thr Thr Ser Gln Pro Ser Leu Ser Ala Ile Val Ala Ala Phe	
775	780 785
gcg cac acc cgt cgc gat acc ggt ttg agc ctc gag gct gtt tct gac	2576
Ala His Thr Arg Arg Asp Thr Gly Leu Ser Leu Glu Ala Val Ser Asp	
790	795 800
ctc gag ccg tac tgg gaa gca gtg cgc gga ctg tac ctg cca ttt gag	2624

Leu Glu Pro Tyr Trp Glu Ala Val Arg Gly Leu Tyr Leu Pro Phe Glu
 805 810 815 820

tct gga acc cca ggc cca acc ggt cgc gtc tac cgc cac gaa atc cca 2672
 Ser Gly Thr Pro Gly Pro Thr Gly Arg Val Tyr Arg His Glu Ile Pro
 825 830 835

ggc gga cag ttg tcc aac ctg cgt gca cag gcc acc gca ctg ggc ctt 2720
 Gly Gly Gln Leu Ser Asn Leu Arg Ala Gln Ala Thr Ala Leu Gly Leu
 840 845 850

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atg ctg gga cgc cca acc aag gtc acc cca tcc tcc aag gtt gtt ggc 2816
 Met Leu Gly Arg Pro Thr Lys Val Thr Pro Ser Ser Lys Val Val Gly
 870 875 880

gac ctc gca ctc cac ctc gtt ggt gcg ggt gtg gat cca gca gac ttt 2864
 Asp Leu Ala Leu His Leu Val Gly Ala Gly Val Asp Pro Ala Asp Phe
 885 890 895 900

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 Ala Ala Asp Pro Gln Lys Tyr Asp Ile Pro Asp Ser Val Ile Ala Phe
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gaa gtt cct gag gaa gag cag gcg cac ctc gac gct gat gat tcc aag 3056
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 950 955 960

gaa cgt cgc aat agc ctc aac cgc ctg ctg ttc ccg aag cca acc gaa 3104
 Glu Arg Arg Asn Ser Leu Asn Arg Leu Leu Phe Pro Lys Pro Thr Glu
 965 970 975 980

gag ttc ctc gag cac cgt cgc cgc ttc ggc aac acc tct gcg ctg gat 3152
 Glu Phe Leu Glu His Arg Arg Arg Phe Gly Asn Thr Ser Ala Leu Asp
 985 990 995

gat cgt gaa ttc ttc tac ggc ctg gtc gaa ggc cgc gag act ttg atc 3200

Asp Arg Glu Phe Phe Tyr Gly Leu Val Glu Gly Arg Glu Thr Leu Ile
 1000 1005 1010

cgc ctg cca gat gtg cgc acc cca ctg ctt gtt cgc ctg gat gcg atc 3248
 Arg Leu Pro Asp Val Arg Thr Pro Leu Leu Val Arg Leu Asp Ala Ile
 1015 1020 1025

tct gag cca gac gat aag ggt atg cgc aat gtt gtg gcc aac gtc aac 3296
 Ser Glu Pro Asp Asp Lys Gly Met Arg Asn Val Val Ala Asn Val Asn
 1030 1035 1040

ggc cag atc cgc cca atg cgt gtg cgt gac cgc tcc gtt gag tct gtc 3344
 Gly Gln Ile Arg Pro Met Arg Val Arg Asp Arg Ser Val Glu Ser Val
 1045 1050 1055 1060

acc gca acc gca gaa aag gca gat tcc tcc aac aag ggc cat gtt gct 3392
 Thr Ala Thr Ala Glu Lys Ala Asp Ser Ser Asn Lys Gly His Val Ala
 1065 1070 1075

gca cca ttc gct ggt gtt gtc acc gtg act gtt gct gaa ggt gat gag 3440
 Ala Pro Phe Ala Gly Val Val Thr Val Thr Val Ala Glu Gly Asp Glu
 1080 1085 1090

gtc aag gct gga gat gca gtc gca atc atc gag gct atg aag atg gaa 3488
 Val Lys Ala Gly Asp Ala Val Ala Ile Ile Glu Ala Met Lys Met Glu
 1095 1100 1105

gca aca atc act gct tct gtt gac ggc aaa atc gat cgc gtt gtg gtt 3536
 Ala Thr Ile Thr Ala Ser Val Asp Gly Lys Ile Asp Arg Val Val Val
 1110 1115 1120

cct gct gca acg aag gtg gaa ggt ggc gac ttg atc gtc gtc gtt tcc 3584
 Pro Ala Ala Thr Lys Val Glu Gly Gly Asp Leu Ile Val Val Val Ser
 1125 1130 1135 1140

taa acctttctgt aaaaagcccc gcgtcttcct catggaggag gcggggccttt 3637

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<212> PRT

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35 40 45
Ser Phe His Arg Ser Phe Ala Ser Glu Ala Val Arg Ile Gly Thr Glu
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Gly Ser Pro Val Lys Ala Tyr Leu Asp Ile Asp Glu Ile Ile Gly Ala
65 70 75 80
Ala Lys Lys Val Lys Ala Asp Ala Ile Tyr Pro Gly Tyr Gly Phe Leu
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Ser Glu Asn Ala Gln Leu Ala Arg Glu Cys Ala Glu Asn Gly Ile Thr
100 105 110
Phe Ile Gly Pro Thr Pro Glu Val Leu Asp Leu Thr Gly Asp Lys Ser
115 120 125
Arg Ala Val Thr Ala Ala Lys Lys Ala Gly Leu Pro Val Leu Ala Glu
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Ser Thr Pro Ser Lys Asn Ile Asp Glu Ile Val Lys Ser Ala Glu Gly
145 150 155 160
Gln Thr Tyr Pro Ile Phe Val Lys Ala Val Ala Gly Gly Gly Gly Arg
165 170 175
Gly Met Arg Phe Val Ala Ser Pro Asp Glu Leu Arg Lys Leu Ala Thr
180 185 190
Glu Ala Ser Arg Glu Ala Glu Ala Ala Phe Gly Asp Gly Ala Val Tyr
195 200 205
Val Glu Arg Ala Val Ile Asn Pro Gln His Ile Glu Val Gln Ile Leu
210 215 220
Gly Asp His Thr Gly Glu Val Val His Leu Tyr Glu Arg Asp Cys Ser
225 230 235 240
Leu Gln Arg Arg His Gln Lys Val Val Glu Ile Ala Pro Ala Gln His
245 250 255
Leu Asp Pro Glu Leu Arg Asp Arg Ile Cys Ala Asp Ala Val Lys Phe
260 265 270
Cys Arg Ser Ile Gly Tyr Gln Gly Ala Gly Thr Val Glu Phe Leu Val
275 280 285
Asp Glu Lys Gly Asn His Val Phe Ile Glu Met Asn Pro Arg Ile Gln
290 295 300
Val Glu His Thr Val Thr Glu Glu Val Thr Glu Val Asp Leu Val Lys
305 310 315 320
Ala Gln Met Arg Leu Ala Ala Gly Ala Thr Leu Lys Glu Leu Gly Leu
325 330 335
Thr Gln Asp Lys Ile Lys Thr His Gly Ala Ala Leu Gln Cys Arg Ile
340 345 350
Thr Thr Glu Asp Pro Asn Asn Gly Phe Arg Pro Asp Thr Gly Thr Ile
355 360 365
Thr Ala Tyr Arg Ser Pro Gly Gly Ala Gly Val Arg Leu Asp Gly Ala

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Lys Met Thr Cys Arg Gly Ser Asp Phe Glu Thr Ala Val Ala Arg Ala		
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Gln Arg Ala Leu Ala Glu Phe Thr Val Ser Gly Val Ala Thr Asn Ile		
	420	425
Gly Phe Leu Arg Ala Leu Leu Arg Glu Glu Asp Phe Thr Ser Lys Arg		
	435	440
Ile Ala Thr Gly Phe Ile Ala Asp His Pro His Leu Leu Gln Ala Pro		
	450	455
Pro Ala Asp Asp Glu Gln Gly Arg Ile Leu Asp Tyr Leu Ala Asp Val		
465	470	475
Thr Val Asn Lys Pro His Gly Val Arg Pro Lys Asp Val Ala Ala Pro		
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Ile Asp Lys Leu Pro Asn Ile Lys Asp Leu Pro Leu Pro Arg Gly Ser		
	500	505
Arg Asp Arg Leu Lys Gln Leu Gly Pro Ala Ala Phe Ala Arg Asp Leu		
	515	520
Arg Glu Gln Asp Ala Leu Ala Val Thr Asp Thr Thr Phe Arg Asp Ala		
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His Gln Ser Leu Leu Ala Thr Arg Val Arg Ser Phe Ala Leu Lys Pro		
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Ala Ala Glu Ala Val Ala Lys Leu Thr Pro Glu Leu Leu Ser Val Glu		
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Ala Trp Gly Gly Ala Thr Tyr Asp Val Ala Met Arg Phe Leu Phe Glu		
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Asp Pro Trp Asp Arg Leu Asp Glu Leu Arg Glu Ala Met Pro Asn Val		
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Asn Ile Gln Met Leu Leu Arg Gly Arg Asn Thr Val Gly Tyr Thr Pro		
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Tyr Pro Asp Ser Val Cys Arg Ala Phe Val Lys Glu Ala Ala Ser Ser		
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Gly Val Asp Ile Phe Arg Ile Phe Asp Ala Leu Asn Asp Val Ser Gln		
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Met Arg Pro Ala Ile Asp Ala Val Leu Glu Thr Asn Thr Ala Val Ala		
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Glu Val Ala Met Ala Tyr Ser Gly Asp Leu Ser Asp Pro Asn Glu Lys		
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Leu Tyr Thr Leu Asp Tyr Tyr Leu Lys Met Ala Glu Glu Ile Val Lys		
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Ser Gly Ala His Ile Leu Ala Ile Lys Asp Met Ala Gly Leu Leu Arg		
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Pro Ala Ala Val Thr Lys Leu Val Thr Ala Leu Arg Arg Glu Phe Asp		
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Thr Tyr Phe Ala Ala Ala Gln Ala Gly Ala Asp Ala Val Asp Gly Ala		

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Val Ala Ala Phe Ala His Thr Arg Arg Asp Thr Gly Leu Ser Leu Glu		
785	790	795
Ala Val Ser Asp Leu Glu Pro Tyr Trp Glu Ala Val Arg Gly Leu Tyr		
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Leu Pro Phe Glu Ser Gly Thr Pro Gly Pro Thr Gly Arg Val Tyr Arg		
820	825	830
His Glu Ile Pro Gly Gly Gln Leu Ser Asn Leu Arg Ala Gln Ala Thr		
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Ala Leu Gly Leu Ala Asp Arg Phe Glu Leu Ile Glu Asp Asn Tyr Ala		
850	855	860
Ala Val Asn Glu Met Leu Gly Arg Pro Thr Lys Val Thr Pro Ser Ser		
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Lys Val Val Gly Asp Leu Ala Leu His Leu Val Gly Ala Gly Val Asp		
885	890	895
Pro Ala Asp Phe Ala Ala Asp Pro Gln Lys Tyr Asp Ile Pro Asp Ser		
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Val Ile Ala Phe Leu Arg Gly Glu Leu Gly Asn Pro Pro Gly Gly Trp		
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Pro Glu Pro Leu Arg Thr Arg Ala Leu Glu Gly Arg Ser Glu Gly Lys		
930	935	940
Ala Pro Leu Thr Glu Val Pro Glu Glu Glu Gln Ala His Leu Asp Ala		
945	950	955
Asp Asp Ser Lys Glu Arg Arg Asn Ser Leu Asn Arg Leu Leu Phe Pro		
965	970	975
Lys Pro Thr Glu Glu Phe Leu Glu His Arg Arg Arg Phe Gly Asn Thr		
980	985	990
Ser Ala Leu Asp Asp Arg Glu Phe Phe Tyr Gly Leu Val Glu Gly Arg		
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Glu Thr Leu Ile Arg Leu Pro Asp Val Arg Thr Pro Leu Leu Val Arg		
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Leu Asp Ala Ile Ser Glu Pro Asp Asp Lys Gly Met Arg Asn Val Val		
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Ala Asn Val Asn Gly Gln Ile Arg Pro Met Arg Val Arg Asp Arg Ser		
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Val Glu Ser Val Thr Ala Thr Ala Glu Lys Ala Asp Ser Ser Asn Lys		
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Gly His Val Ala Ala Pro Phe Ala Gly Val Val Thr Val Thr Val Ala		
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Glu Gly Asp Glu Val Lys Ala Gly Asp Ala Val Ala Ile Ile Glu Ala		
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Met Lys Met Glu Ala Thr Ile Thr Ala Ser Val Asp Gly Lys Ile Asp		
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<223> Description of Artificial Sequence: PCR primer

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19

<210> 4
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19